**FINAL REPORT FOR 2021 GRANT**

**1. Project Title**
Pharmacological Degraders for the Cellular Prion Protein

**2. Project objective.** Compelling evidence indicates that decreasing the cellular prion protein (PrP) could lead to substantial therapeutic benefits in prion disease patients. We have developed an approach for selectively reducing the level of target proteins by promoting their degradation during the folding process. This method, called Pharmacological Protein Inactivation by Folding Intermediate Targeting (PPI-FIT), is made possible by computational algorithms allowing the full atomistic reconstruction of folding and misfolding processes of polypeptides. Thanks to PPI-FIT, we have identified SM875, a promising molecule able to reduce PrP levels and block prion replication in cells. However, the compound must be further developed to improve its pharmacological properties before it can safely be administered to humans. The project intends to directly pursue these tasks, with the ultimate goal of defining a novel pharmacological option for prion disease patients.

**3. Summary of accomplishments to date.** As a first step in the optimization process, we have improved the chemical synthesis and the purification scheme to achieve larger quantities of SM875 and analogues in a shorter time. Next, we developed a cell-based assay suitable for quickly evaluating SM875 effects. Thanks to these experimental advances, we have currently synthesized, purified, structurally characterized and tested over 40 SM875 analogues. Most of these molecules showed equal or decreased PrP-suppressing activity compared to the parent compound, while very few analogues showed moderately improved effects. These results are not surprising, as a typical chemical optimization process may require the synthesis and test of hundreds of analogues. Notably, the data already provide information to start defining a structure-activity relationship for the compound, which is currently guiding us in designing new and more focused analogues. At the end of such an iterative process, we aim to identify an analogue showing a strongly improved effect.

**4. Key findings and implications for the prion disease field.** Over the last ten years, multiple research groups, including ours, have tried to target PrP at various levels. These include ablating its gene with the CRISPR-Cas9 technology, decreasing its synthesis with specific antisense oligonucleotides, or designing drugs that bind directly to PrP or alter its localization. As a result, several chemical scaffolds have been claimed to exert anti-prion effects by targeting PrP. Unfortunately, most of these molecules show inconsistencies between affinity for the protein and biologically-active concentrations, low binding specificity, or lack of reproducibility. As a result, not a single approach appears as an immediate candidate for clinical testing in prion diseases soon, except for an antisense oligonucleotide (ASO) against PrP, which may enter the clinical trial in 2023. The negative success rate in the search for prion therapeutics forced us to explore new non-canonical strategies to lower PrP expression. The result was the development of PPI-FIT, a technology now exploited by a company named Sibylla Biotech in many other disease fields. Our application of PPI-FIT to suppress PrP is currently in the so-called hit-to-lead phase, a tedious, lengthy, but fundamental step along the drug development pipeline necessary to bring a candidate drug closer to the clinical setting. The results collected so far are highly encouraging and provide strong support for the objective of identifying a drug-like candidate capable of suppressing PrP in vivo.

**5. Next steps in your work (or other work you're doing in the field if you'd like to share it).**
We plan to continue the chemical optimization process until a suitable SM875 analogue is identified. The molecule will then be evaluated for other important pharmacological properties relevant for the use in vivo (for example, the ability to penetrate the brain) and eventually further modified to achieve an ideal candidate drug. The therapeutic potentials of such a compound will then be evaluated in mouse models of prion diseases, a preliminary step toward its employment in clinical trials.